RESPIRATION DURING CELL DIVISION IN THE EGG OF THE SEA URCHIN PSAMMECHINUS MILIARIS

ERIK ZEUTHEN [1]

Laboratory of Zoophysiology, University of Copenhagen, Denmark, and Kristinebergs Zoological Station, Sweden

Introduction

In a previous paper (Zeuthen, 1946) it was reported that during early development of the egg of the frog (*Rana plathyrrhina*) each cell division is accompanied by a slight increase in oxygen uptake. The curve describing oxygen uptake as a function of time roughly approximates a sine curve. The division furrow of the first, second, and third cleavages always appears during a period of increase in oxygen uptake.

The experiments were performed in modified Cartesian divers. The rate of decrease in pressure in an oxygen bubble situated close to the egg was measured. The respiratory carbon dioxide, however, was not chemically absorbed, but held in solution by the egg and by a considerable volume of water bathing the egg. The diver system, of course, was not shaken, so that all exchange of gases had to take place over certain diffusion distances.

Both factors mentioned, *viz.:* the incomplete separation of O_2 and of CO_2 in the diver and the diffusion distances present in the system, make it somewhat doubtful how to evaluate the results. Linderstrøm-Lang (1946) made elaborate calculations on the results obtained by Zeuthen and concluded that the observed waves are probably slightly delayed and somewhat damped as compared with the curves which would describe the true respiratory processes in the egg itself. According to Linderstrøm-Lang the wave amplitude found (about 5 per cent of the respiratory rate in the minima between the waves) would probably indicate "true waves in the egg" with a maximum amplitude of 7–11 per cent of the respiration in the minima.

These experiments do not quite conform with those of Brachet (1934, 1935) working with the Fenn-respirometer. He found two considerably larger variations in respiratory rate per mitosis in the egg of *Rana fusca.* The discrepancy between the present author's experiments and those of Brachet remains unexplained. Brachet has expressed the hope of being able to repeat his experiments (1945).

In a recent letter in *Nature,* Tang (1948) claims many years ago (Tang, 1937) to have found slight mitotic variations in oxygen uptake in the sea urchin egg, however only at a high temperature (25° C.). Tang considers that the failure of Gray (1924) to find any change in oxygen uptake in the dividing sea urchin egg is due to the fact that the experiments were performed at a low temperature (11° C.). Tang as well as Gray used the Warburg technique. Unfortunately, Tang has not been able to supply a copy of the original paper which was published

[1] Permanent address: Laboratory of Zoophysiology, University of Copenhagen.

in a Chinese journal. All records were destroyed during the war. Also Runnström (1934) found slight fluctuations in respiration in dividing sea urchin eggs. According to this author there is a maximum of respiration during prophase.

None of the experiments mentioned, however, have appeared convincing to the reviewers in the field. The problem of respiration during mitosis is not solved yet. Linderstrøm-Lang's calculations on Zeuthen's experiments on the frog egg indicated that in future experiments with the Cartesian diver we would have to employ more intensely respiring eggs than the frog's egg, and we would have to secure complete carbon dioxide absorption in the diver. Both conditions are fulfilled in diver experiments on eggs of the sea urchin *Psammechinus miliaris* to be reported in this paper (a short note on this work has already been published in *Nature,* 1947). The curve found for this egg must therefore be considered rather accurately to describe the true processes in the egg itself. The experiments on *Psammechinus* are concentrated on later divisions (nos. 4–8), and the main object of the investigation is to establish possible mitotic variations in the respiration of this egg. The experiments were carried out in July–Aug., 1946. In a following paper on the egg of *Urechis* the first three or four divisions are considered, and on this object the exact time relations between respiration and stage of mitosis are given.

Material and Methods

Eggs of *Psammechinus miliaris* (Z-form) were fertilized artificially. It was not always possible to obtain batches of eggs which divided 100 per cent and synchronously, but if those eggs which were the first to enter the first division were picked out with a braking pipette and kept separately, these eggs invariably were found to divide well (95–100 per cent) and synchronously (i.e., within 5 min.) through a series of successive divisions. For each experiment 300–400 eggs were isolated in this way, the jelly was removed with a pipette, and about 200 eggs were transferred to the diver; the rest were kept as controls outside the diver in the same thermostat. In the diver the eggs were densely packed, but they were very close to a bubble of oxygen. The oxygen supply was calculated to be ample. Usually the first 5–6 divisions could be observed in the diver itself (not on all eggs, but on a representative number of eggs). In some cases, however, and especially for later divisions, I depended on observations of the controls in determining the cleavage times in the divers. In earlier stages, where comparisons could be made between division rates in-and-outside the divers, the rate of development was found to be uninfluenced by the stay in the diver.

The way of securing egg material which divided synchronously restricted this investigation to the later stages of development. The divisions nos. 4–8 were studied, these divisions taking the egg from the 8-cell to the 256-cell stage.

Two types of divers are used—neither of them very different from the one previously described (Zeuthen, 1950a). Both float with the stopper upwards, however, so that the eggs lie on the bottom of the diver, separated from the air space by a thin layer of water. The distance from the most remote eggs to the air space did not exceed 0.7 mm. in any experiment. The diver which is shown in Figure 1-A is the most sensitive because it contains a very small oxygen bubble (about 0.2 μl.). This air bubble is not large enough to separate the neck fluid from the bottom fluid.

The fluid which fills the neck and bathes the eggs is a CO_2 binding buffer: glycylglycine m/10 or m/50 in sea water, with pH adjusted to 8.2 by the addition of NaOH (pK of the amino group in glycylglycine being 8.07 according to Schmidt (1938, p. 613)). The buffer solution is made isotonic with sea water. During the division periods with which we are concerned (Fig. 2), the m/50 buffer was not observed to influence the development of the eggs at all, but in the m/10 buffer a slight retardation of the later cleavages (7–8) was noticed, and the blastulae did not swell quite normally. In the diver the oxygen bubble was kept down, close to the eggs, by the aid of a tiny glass extension on the stopper. The diver shown has the exact dimensions of the diver used in the experiment reproduced in Figure 2.

The diver shown in Figure 1-B is almost identical with the one used for the experiments with *Urechis,* the main difference being that it floats with the stopper upwards. Figure 1-B gives the exact dimensions of the diver which was used in

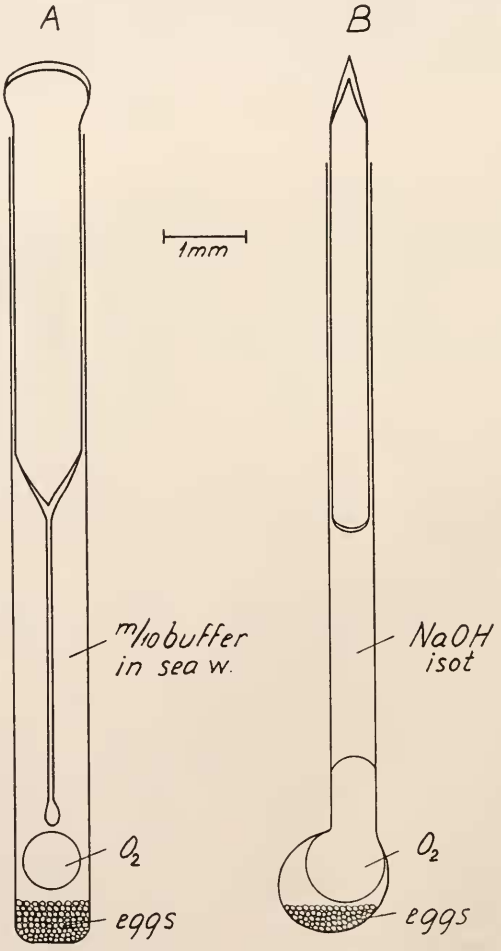

Figure 1. The divers used.

all three experiments of Figure 3. The air space (0.93 μl.) separates the bottom fluid with the eggs from the isotonic NaOH neck fluid. The diver floats in isotonic NaOH. Only the later cleavages (7–8) and the hatching period in *Psammechinus* have been studied with this diver. The accuracy of these experiments is not so high as it is now obtained in experiments with *Urechis*. Probably the temperature control in the thermostat (running sea water) could have been better.

Both types of divers were filled with pure oxygen. During the first part of the experiment, O_2 is used for saturating the fluids inside the diver. As mentioned elsewhere (Zeuthen, 1950a, c), this tends to make the measurements too high in the first part of the experiment. A possible respiratory rhythm should manifest itself even during this early period, however.

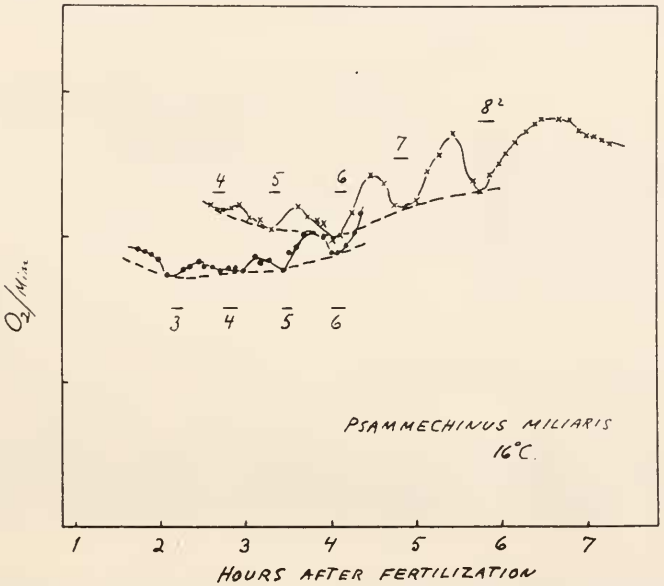

FIGURE 2. Two experiments with the diver shown in Figure 1-A, buffer m/10 glycylglycine.

The CO_2 absorption in both groups of experiments is considered complete. Since, moreover, the distance between the most remote eggs and the air bubble is small in all experiments, the damping of the respiratory waves found may amount to 5 per cent only, and the delay with which they are measured to less than two minutes. This can be determined from the tables in the paper of Linderstrøm-Lang (1946). Both the damping and the delay mentioned are insignificant, considering the not too high accuracy with which the dimensions of the respiratory waves can be recorded.

The temperature of the bath was regulated at about 16° by rapidly running sea water. The equilibrium pressure was determined as described by Zeuthen, 1946, 1950a (turning point determinations). The apparatus used was built in a very simple way at the Zoological Station, as far as possible following the directions of Holter (1943).

Experiments

Figure 2 shows two experiments with the diver of Figure 1-A. The ordinate gives the respiratory rate in terms of change (in cm. Brodie) per minute of the equilibrium pressure of the diver. The abscissa gives the time in hours after fertilization at 16° C., the 5th cleavage occurring 3 hours, 20 minutes after fertilization.[2] Each measurement is plotted in the middle of the period for which it is valid.

After the initial period with too high readings is over, the true slope of the two curves is indicated. The main trend of the curves seems to conform fairly well with the S-shaped curve of Lindahl (1936) showing the increase of respiration of

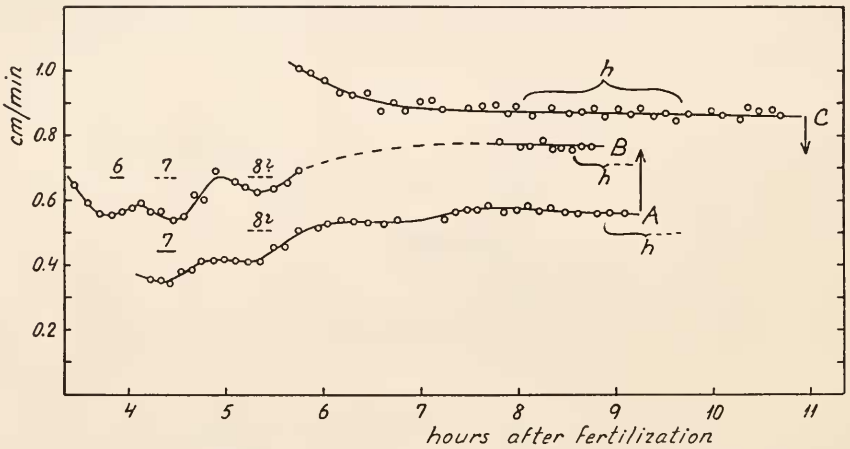

Figure 3. Three experiments with the diver shown in Figure 1-B. Actually all three curves should have been plotted at the same level as curve B. For the sake of clearness the curves have been separated in space. The arrows indicate the displacement. *h* indicates the hatching period.

the sea urchin egg during the cleavage period. However, waves are superimposed on this curve, one wave per mitosis. The amplitude of the waves increases as development proceeds and as more and more simultaneous divisions occur in the embryos. The cytoplasm cleaves during the period of decreasing respiration, in some cases perhaps even when the respiration is at a minimum.

All experiments of Figure 3 were performed with the diver shown in Figure 1-B. These experiments differ from those of Figure 2 in that the eggs lay in

[2] Dr. Hans Borei, Stockholm, has pointed out to me that the time relations on my curve in *Nature* are not quite correct (Borei, 1948). In *Psammechinus* the 5th cleavage (at 16° C.) appears 3 hours, 20 min. after fertilization, not after 4 hours, 30 min., as indicated in my figure. I have not myself measured the time (at 16° C.) from fertilization to start of the single experiment, because the necessary manipulations during this period involved serious temperature changes. From this and other reasons the said curve was only intended to give a rough survey to indicate for the reader on which part of the respiratory curve in the sea urchin the respiratory waves are located. However, I thank Dr. Borei very much for his information which I have taken advantage of in timing the curves of Figures 2 and 3 of this paper in relation to the time of fertilization.

natural sea water throughtout the experiment, CO_2 being chemically absorbed by the alkaline neck fluid. The curves of this figure form a continuation of those of Figure 2. The eggs were introduced into the divers at the 64-cell stage or even later, and in one experiment (C) the respiration was followed until hatching of all eggs had taken place in the diver. Saturation equilibria are not established until about 1 hour after start of experiment. Cleavages 6 to 8 seem to be accompanied by large variations in respiratory rate; later the respiratory variations again become slower and slighter, and for a long time after, no variations whatsoever in respiratory activity were disclosed, despite the fact that all the larvae hatched one by one during this period.

The general trend of Figure 3: a continued rise in metabolic rate followed by a period of constant respiration during the period of hatching conforms again with Lindahl's S-shaped curve—in this case with the later half of the S.

Discussion

The experiments indicate rather conclusively that the mitoses in the sea urchin egg are accompanied by changes in respiratory rate. But the interpretation remains uncertain in this respect: Is the increase or the decrease in respiration the important thing? As a matter of convenience, we shall in this paper interpret the curves as indicating that each mitosis is accompanied by a slight excess oxygen uptake. The respiration increases at some early stage of the mitosis, attains a maximum and decreases again at the end of the mitosis when the cytoplasm cleaves.

A smooth auxiliary line, which just touches all minima of the experimentally found curve, will separate a number of small areas above the auxiliary line from a large area below this line. According to the accepted point of view the large area below the line would indicate some kind of "basal metabolism" of the eggs, whereas the small areas would measure the "excess metabolism" of the individual mitoses.

The upper curve of Figure 2 shall now be analyzed in detail in order that we can get some idea of the relative magnitude of the "basal" and of the "excess" metabolism of the division cycle numbers 5–8 incl. in the egg of *Psammechinus*. There is a difficulty here because the measured respiration is known to be too high in the initial period of the experiment (see above). In this particular experiment, however, the diver had been in the flotation vessel for about ¾ of an hour before measurements were taken. Calculation by the formulae of Linderstrøm-Lang (1946) indicate that after about another 1.5 hour the initial period of this particular diver (dimensions fig. 1-A) is practically over. The curve found seems to agree well with this view. After the 6th division the general trend of the curve conforms well with the curve of Lindahl. In the upper curve of Figure 2 we may therefore introduce a reasonable correction for the measured respiration in the first part of the experiment by extrapolating the curve found after the 6th division back to the time of 5th and 6th division. In this way we find that the respiration was approximately 25 per cent too high during the 5th division and about 10 per cent too high during the 6th division. This way of correcting is admittedly rather arbitrary, but in view of the comparatively low accuracy of the whole analysis, it is probably satisfactory.

Table 1 gives the results of the analysis.

TABLE 1

Division number	Number of cells dividing per embryo	Wave area	
		Absolute	Percentage
5	16	1.0	3.4
6	32	2.1	5.0
7	64	3.9	7.7
8	128	5.7	9.6

The different areas between curve and auxiliary line touching all the valleys are determined by weighing the areas after cutting the graph paper. In Table 1, column 3, the extra O_2 (absolute measure, independent on correction) taken up during the 5th division is taken as unity and it appears that during the 8th division approximately 6 times as much extra oxygen is consumed as that taken up during the 5th division. As indicated in column 4 the "excess oxygen consumption" is but a small fraction of the basal oxygen consumption in the same period, the figures being 3.4 per cent for the 5th division and 3 times as much (9.6 per cent) for the 8th division. The reason why the percentage extra O_2 increases less than 3-fold at the same time as the absolute extra O_2 increases 6 times is partly due to the fact that the "basal" metabolic rate increases with development, but mostly because also the time occupied by one division increases. It is unknown whether this longer division time in later stages is due to an extension of one or more stages of the mitosis proper, or to the appearance of an interphase. Although only one experiment has been considered good enough to permit a close analysis, qualitatively this experiment is supported by the other experiments published. The two experiments of Figure 2 are in good agreement in indicating the approximate size of the respiratory waves accompanying divisions 4–6. Also, the important experiments of Figure 3 show that the large waves accompanying the later divisions can be found when no artificial buffer systems are added to the sea water. In these experiments the waves were found in the initial period, e.g. before saturation equilibria had become established in the diver. For this reason we shall not try to make a quantitative comparison with the experiments of Figure 2.

The safest and at the same time the most promising conclusion to be drawn on the basis of Table 1 is that for the three successive divisions 5–6–7 there is a doubling of the "excess oxygen consumption" for each new division. Thus, over this period the extra oxygen taken up per mitotic cycle is approximately proportional to the number of cells dividing. This finding is very different from what was found for the first three divisions in *Urechis* (Zeuthen, 1950c) and in the frog (Zeuthen, 1946). In these two eggs the extra oxygen taken up per mitotic cycle is essentially constant despite the fact that the number of dividing cells increases from 1 to 4. In the early divisions the *Psammechinus* egg (comp. the lower curve in Fig. 2) seems to line up with two other eggs mentioned, so probably it is safe to conclude that in dividing eggs some physiological difference exists between the early and the later mitoses.

Table 1 seems to indicate that after the 7th division in the *Psammechinus* egg matters change again. The extra oxygen consumption of the 8th division is only 46 per cent higher than that of the 7th division. Figure 3, curve B, however, shows an 8th respiratory wave which is approximately twice the preceding one. In this experiment no buffer was added. Both figures (Figs. 2–3) agree in indicating a big 9th wave which is very extended in time, though. After this no more respiratory fluctuations could be detected although ciliation and hatching of the embryos took place in the diver. It is well known that the mitotic rhythm characteristic for the dividing egg does not continue forever. The data of Table 1 and the curves of Figures 2 and 3 are perhaps best explained by the assumption that at some time—presumably around the 7th–9th division—the mitoses in the *Psammechinus* egg gradually become significantly asynchronous, less numerous and perhaps restricted to specific areas of the embryo. We are gradually approaching the period of differentiation with which this paper is not concerned.

For the deep interest in the problems involved and for valuable help during the performance of the work at Kristinebergs Zoologiska Station the author is very much indebted to Professor John Runnström, University of Stockholm.

Summary

The later divisions in the egg of *Psammechinus miliaris* are accompanied by variations in oxygen uptake. As in the embryo more and more cells divide simultaneously, the respiratory variations become more and more pronounced. The phenomenon is interpreted as an extra O_2-uptake correlated with the mitosis. During the four divisions nos. 5–8 proportionality is approximated between number of cells dividing and extra O_2 taken up. Thus, for the division period considered, the extra O_2 consumed by a dividing cell approximates constancy, *viz.*: it is independent of the size of the dividing blastomere.

REFERENCES

Brachet, J., 1934. *Arch. f. exp. Zellforsch.,* **15**: 46.
Brachet, J., 1935. *Arch. de Biol.,* **46**: 1.
Brachet, J., 1945. *Embryologie Chimique.* Paris, Liége.
Borei, Hans, 1948. *Biol. Bull.,* **95**: 124.
Gray, J., 1924. *Proc. Cambr. Philos. Soc.* (Biol. Sect.), 1: 164.
Holter, Heinz, 1943. *Compt. rend. Lab. Carlsberg,* Sér. chim., **24**: 399.
Lindahl, P. E., 1936. *Acta Zool.,* **17**: 179.
Linderstrøm-Lang, K., 1946. *Compt. rend. Lab. Carlsberg,* Sér. chim., **25**: 229.
Runnström, J., 1934. *Protoplasma,* **20**: 1.
Schmidt, C. L. A., 1938. The chemistry of the amino acids and proteins, p. 613. Baltimore, Md.
Tang, P. S., 1937. *Wuhan Univ. J. of Science,* **7**: 1.
Tang, P. S., 1948. *Nature,* **162**: 189.
Zeuthen, E., 1946. *Compt. rend. Lab. Carlsberg,* Sér. chim., **25**: 191.
Zeuthen, E., 1947. *Nature,* **160**: 577.
Zeuthen, E., 1950a. Cartesian diver respirometer. *Biol. Bull.,* **98**: 139.
Zeuthen, E., 1950c. Respiration and cell division in the egg of *Urechis caupo*. *Biol. Bull.,* **98**: 152.